Template for Education Applications of Projects Developed by EREN
(Adapted and modified from TIEE -TEACHING ISSUES AND EXPERIMENTS IN ECOLOGY)

Project Title: How many are there? Estimating population size of a freshwater turtle using the mark-recapture technique

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Main Ecological Questions:

1) What is the population size of a given turtle species in a single aquatic habitat?

Secondary Ecological Questions:

1) What is the population structure of a given turtle species in a single aquatic habitat?
   a) How many adult turtles?
   b) What is the adult sex ratio?
   c) How many immature turtles?

Ecological Concepts/Framework:
As stated in most introductory biology and ecology textbooks, one of the chief goals of ecology is to understand the processes influencing the abundance and distribution of organisms. This exercise introduces students to a fundamental technique of how to estimate abundance, namely mark-recapture.

Application:
The data from this activity can be used as a stand alone for curriculum **Yes**/No

The data from this activity will work with data from other EREN sites Yes/**No. As written, this activity is stand alone but can be easily modified to take advantage of the network in order to answer more complex questions.**

Methodology:

- Number of labs required for minimum implementation:
  - Two within the same week. This can be accomplished by incorporating multiple lab sections or if only one lab section exists, having students volunteer to obtain extra experience.
- Number of labs required for optimal implementation:
  - More than two

- Faculty time pre lab preparation and trips to field that might be needed:
  - The pre-lab preparation consists of the one-time investment of purchasing the turtle hoop traps and constructing the trap supports. A day prior to the lab, the professor, hopefully with assistance of

students, will set the traps. Depending on the location of the pond and ease of access, trap setting will take about one hour. Traps will be checked for at least two consecutive days and then removed.

Students' skills

What biological background is needed for students to participate in this activity?

No biological background is needed to participate in this activity. Students will derive the most benefit if they have a basic understanding of turtle biology and population ecology.

What analytical, instrumental, etc skills are needed for students to participate in this activity?

Existing: Basic algebra

Acquired: Students will develop skills in handling wildlife and measuring body dimensions using dial calipers. Students will also learn external turtle anatomy and how that is used to identify both turtle species and individual turtles. Students will then manipulate, analyze, and interpret their data in order to estimate population size.

Outcomes

Student learning outcomes:

1) Perform a basic technique (mark-recapture) to survey a wildlife population
2) Estimate size of a wildlife population from the mark-recapture study using the Lincoln-Petersen Index.
3) Identify basic external turtle anatomy.
4) Identify turtle species and sex
5) Handle and measure individual turtles.

Assessment type

Students will be given pre- and post exercise questions on turtle anatomy, species identification, turtle naming, and techniques on how to estimate population size of cryptic animals.

Skills and learning

Students will become skilled in using dial calipers, handling a wildlife species, turtle species identification, and use of a spreadsheet program (Excel) to enter, manipulate, and calculate population size.

Other measurable outcomes

Critically assess the value of the population estimate by careful consideration of assumptions of the model.

Formulate hypotheses to explain the turtle results (e.g., what is causing population size, structure, and species composition).

Transferability (Links between research and teaching). Research data can use one-lab time versus more lab times and the education piece can use data from the EREN dataset.

This exercise is designed to teach students on how to estimate the size of a wildlife population at a single location. These data, once shared across the network, can then be used to test hypotheses as to what factors influence population size.

Create matrix linking students activities in labs with learning, concepts and skills?

Students Instructions
See document below.

Faculty Notes
Please see directions below on how to set and check traps.

For faculty only. It should include information about

1. specific student-active teaching approaches

The trick with this lab exercise is to get the students to think about what they are doing and why and not treat it as a fun lab to catch turtles, although I hope it is that as well. The students should be encouraged to think about the assumptions of the population estimation methods, factors that may influence trapping success, the reason why some turtle species but not others are found in this habitat, why some trap locations may be successful and others not. The students will not be able to answer these questions with single site data, but the process of formulating hypotheses is an important one. As we start sharing data, students could begin to test their more advanced hypotheses.

The students could also be encouraged to think about how this population estimation technique can be applied to other organisms. What organisms can be studied this way, how could you mark them, do you need the individual in hand or can you simply see them. I like my students to be able to apply this technique to other organisms, including people and plants.

2. tips and strategies that will help faculty anticipate students responses, misunderstandings, misconception, questions for discussion and methods for evaluating student understanding. WRITE THIS SECTION AS IF YOU WERE TALKING DIRECTLY TO A FELLOW FACULTY MEMBER

The one thing that has confused my students is what is meant by a recapture. With a turtle's long life, you will have recaptures from year to year. But in order to satisfy the closed population assumption of the Lincoln-Petersen Index, a recapture is only considered within one year's trapping session. So if ADH was marked in 2010 and then caught again on trap day 2 of 2011, it is not considered a recapture for the purpose of this exercise. It would have had to be caught during trap day 1 of 2011 to be considered a recapture within 2011. This will become more important and confusing as you collect more data over the years. A good way around this is to write the turtle's name with a Sharpie on the plastron. We can treat that as the within year "mark" because it won't last from year to year.

As we start collecting more annual data, we'll need to employ more advanced population estimation techniques that relax the closed population assumption to examine year to year differences.

Challenges to anticipate and solve:
Provide a list of the principal challenges that you encounter with your students with this experiment and describe potential solutions

Some students are hesitant to put on waders and walk into a pond, but always due with encouragement. I stress the importance of moving slowly and deliberately when in the water. It is very different from walking on land. A student tripping in the water is always amusing for the rest of the class but can be dangerous if the waders fill with water. BE SURE THAT NO ONE GOES IN DEEP WATER. DROWNING CAN OCCUR IF THE WADERS FILL.

Students are wary of snapping turtles. Snapping turtles are quite docile in the water so the students have nothing to fear about stepping on them or being bitten while the turtle is in the water. But just to make sure, have students checking the traps pick the traps above the water line. If a snapping turtle is in the trap, just bring the trap to shore, collapse it, and let the snapping turtle free itself. No student should touch a snapping turtle unless he/she has been specifically trained. The neck of a snapping turtle can reach about three-fourths of the distance to the tail, so be sure to keep clear.

If time allows, have multiple students measure the same turtle and confirm the turtle identification. This can serve as data quality control. I often assign a few students to be field assistants and give them advance training. They then help the less experienced ones. This role could be filled by teaching assistants.

The population estimation technique requires recaptures, so if no recaptures occur, one cannot calculate an estimate. I leave this for the students to discover and realize this limitation of the technique. If desired, you can give them a different dataset to run the numbers. I can supply plenty of data.

DATA AVAILABILITY - short – long te**rm**

Until everyone starts sharing data, I will provide sample datasets from my study sites in Pennsylvania and Virginia.

How many are there?

Estimating population size of a freshwater turtle using the mark-recapture technique

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Student Learning Outcomes:

6) Perform a basic technique (mark-recapture) to survey a wildlife population
7) Estimate size of a wildlife population from the mark-recapture study using the Lincoln-Petersen Index
8) Recognize external turtle anatomy
9) Identify turtle species and sex
10) Handle and measure individual turtles

1. Population Estimation

How many cockroaches are in your dorm? You may hope the answer is zero, but in reality it's probably not. A good, reliable estimate of cockroach population size is needed to make a rational decision on what to do about the cockroaches. If you spend money on an exterminator, how will you know if it was worth the effort? Answering that question requires a second estimate of population size, one measured after the management effort. The cockroach example is meant to 1) cause some of you to go "Ew, that's gross" and 2) illustrate one of the most fundamental of ecological questions. How many of species X exist at a particular location is the essential starting place to know if species X is increasing, staying the same, or decreasing toward extinction. One can then investigate what factors are influencing that change in population size. Is it a change in predation, disease, competition, or some resources?

The answers to those more advanced questions depend on one's ability to calculate population size. This may be straightforward for large, obvious, relatively sedentary organisms like trees. But most organisms are hidden, small, and tend to move around. Think back to the cockroach example. You can't just count cockroaches because most individuals are hidden and those seen could be double counted. For these and other reasons, animal ecologists rarely directly count all individuals to determine population size. Instead, they use a technique to **estimate** population size. In this exercise, you will learn one of the most basic techniques (mark-recapture) and mathematical indexes (Lincoln-Petersen) to estimate population size.

Mark-recapture and the Lincoln-Petersen Index

A mark-recapture study consist of capturing individuals, putting a unique identifier on each (ie., the mark), releasing them back into the wild, and then trying to capture individuals again at some later time. Some of the individuals caught at time 2 will have already been captured at time 1 (ie. the recapture), but some will be new captures. By examining the ratio of recaptures to new captures, one can estimate overall population size. The simplest means to do this is by the Lincoln-Petersen index:

$m_2/n_2 = m_1/N$, where

m_1 = the number of individuals caught, marked, and released during the first trapping period

m_2 = the number of marked individuals in the second trapping period (the recaptures)

n_2 = the total number of individuals caught in the second trapping period

N = the estimate of the total population size.

You also want to know how good your estimate is of the actual population size. You can calculate standard error (SE), which is a characterization of how close your population estimate is to the real population size (Samuels and Witmer 2003), with the following formula:

Standard Error (SE) =

$\text{Sqrt}\ [(m_1^2\ n_2(n_2-m_2)/m_2^3]$ (Davis and Winstead 1980).

Here's an example using cockroaches. Let's say that you set some traps in your dorm that captures but does not kill the insects. You check the traps on Monday and capture 125 individuals. You then mark each one and set them free to roam around your dorm. On Tuesday, you check the traps again and catch a total of 100 individuals, with 75 of them being marked (ie., recaptured). Use these data to estimate N to the closest integer and then calculate SE.

m_2 = __________

n_2 = __________

m_1 = __________

N = ___________

SE = ___________

For this index to be valid as an estimate of the population size, the following assumptions must be met:

1) Closed population, meaning that the population size does not change over the study period.
2) Each individual has an equal probability of capture. Individuals that have already been caught must not have learned to avoid the traps.
3) The identifying mark is not lost over the study period and does not influence survivorship.
4) The population is well mixed, meaning that marked and unmarked individuals freely circulate within the habitat.

With a partner, discuss how violations of each assumption could impact your population estimate. Write your answers below and prepare for a class discussion on the topic.

2. Estimating Population Size of a Turtle Species

You and you classmates will soon attempt to estimate the population size of turtles living in a local lentic ecosystem. You will also estimate the number of adult males, adult females, and immature turtles. In order to accomplish this, you will collect the following information.

Write the date and pond name at the top of the provided datasheet. For each capture, record the following on a unique row in the datasheet:

- Last name of the person measuring the turtle (trapper)
- Last name of the person recording the data (recorder)
- Status of the turtle (new capture, recapture, or recapture but identity is uncertain)
- Turtle identification code (usually 3 letters, see below)
- Species
- Length of carapace, to the nearest 0.1 mm (see directions below)
- Length of plastron, to the nearest 0.1 mm (see directions below)
- Pre-cloacal tail length, to the nearest 0.5 mm (see directions below)
- Right middle foreclaw length, to the nearest 0.1 mm (see directions below)
- Annuli, the number of growth rings on the right abdominal scute (see directions below)
- Comments on unusual characteristics of the turtle

Before you start collecting these data, you must first learn how to safely handle turtles, identify turtle species, measure turtles, determine sex and age class, and mark individuals. Pay close attention to the following instructions.

A. Safe turtle handling

CAUTION: Turtles are known carriers of *Salmonella*. Be sure to wash hands before handling food or drink. You are advised to wear disposable examination gloves, but this is not required as long as good hygiene practices are strictly followed.

CAUTION: Turtles may bite and scratch. Turtles, aside from snapping turtles, are unlikely to bite but it is possible. The best way to avoid being bitten is to keep one's fingers away from the turtle's mouth. It is quite easy to dangle one's fingers in front of the turtle's head especially when measuring the turtle's length. Pay attention to this and consciously keep the fingers tucked in. The claws of turtles are quite sharp and one can be easily scratched. Wearing gloves is the easiest way to protect oneself.

B. Species identification

Turtle species can be differentiated by differences in the size, shape, and coloration of the shell, head, and neck. Your instructor will provide you more details on how to identify turtles in your area.

C. Measuring turtles

The most obvious feature of a turtle is its shell. The top (dorsal) shell is called the carapace and the bottom (ventral) shell is the plastron. The carapace and plastron are each comprised of segments called scutes (Figure 1 and 3). On the carapace, the scutes along the edge are the marginal scutes, the ones down the middle are the vertebral, and the ones in between are the costal scutes (Figure 1). The number and arrangement of scutes are usually consistent within a species and you'll use this information to help mark and identify individual turtles. Some individuals will have extra scutes and you are to take note of any abnormalities in scute number or arrangement.

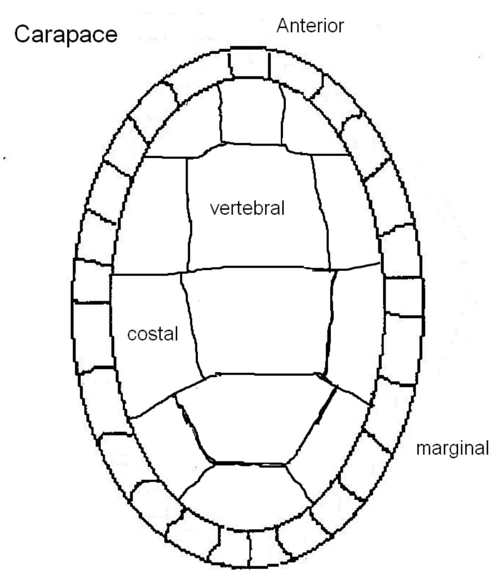

Figure 1. Location and arrangement of scutes of the carapace

Carapace and plastron: We will measure the straight-line (anterior to the posterior) distance of each shell using dial calipers (Figure 2). Your instructor will demonstrate how to use the dial calipers.

Figure 2. Measuring with dial calipers the plastron of a painted turtle.

Pre-cloacal tail length: Near the base of the tail is the cloacal opening. The distance from the base of the tail to this opening is useful in determining the sex of the species – typically, this length is greater in males. Use the dial caliper to measure this distance.

The pre-cloacal tail length measurement depends on how cooperative the turtle is being and as such may not be very accurate. It's more a relative measure of long versus short but measuring it with the calipers gives an objective way of determining that. For example with a painted turtle, if the true distance is 16.5 mm but you measure 15 mm, that measurement still demonstrates it's a long precloacal tail and therefore a male. An adult female would be closer to 7 mm.

Right middle foreclaw: The length of the nails is useful for many species in determining both sex and maturity. Males tend to have longer nails than females and sexually mature males have longer nails than do juvenile males. For painted turtles, the claw length of ≥10 mm indicates sexual maturity in males only (Frazer et al., 1993). Use the dial caliper to measure this distance.

Annuli: The annuli (growth rings) of turtles can be useful in determining age for the first several years of life. If annuli are visible on the abdominal scute of the plastron, record their number (Figure 3).

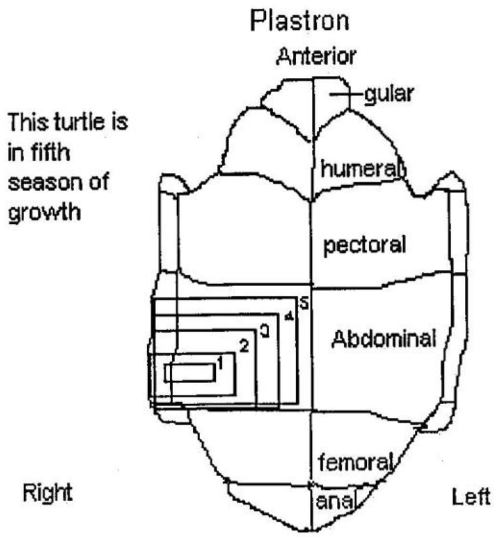

Figure 3. Schematic of annuli and scutes on the plastron of a turtle and photograph of the plastron of a juvenile eastern painted turtle (notice its annuli). The writing on the plastron is this turtle's identification code. The writing is not permanent but does ease data collection on recent recaptures

D. Determining Age/Sex Class

The species, age, and sex of the turtle will be judged from a variety of physical measurements. Most turtle species are sexually dimorphic but the specific characteristics vary by species. The most likely species to be caught in campus ponds are the so-called "basking" turtles, so named for their habit of sunning on logs. These species tend to be size dimorphic, with adult females being larger than adult males. Adult males, however, tend to have longer foreclaws and a longer distance from posterior of plastron to the cloacal opening. I recommend using a field guide such as "A Field Guide to Reptiles & Amphibians of Eastern & Central North America (Peterson Field Guide Series)" to assist you in identifying species and age/sex class.

Here are ways to determine age/sex class (Freedberg and Bowne 2006) for the most common species of basking turtle, the painted turtle (*Chrysemys picta*).

Adult male:

A front middle claw length of ≥10 mm indicates sexual maturity in males only (Frazer et al., 1993). The carapace and plastron of adult males are shorter than adult females but the pre-cloacal tail length is much longer, often twice as long.

Adult female:

Plastron length exceeds 118 mm. They have shorter claws and shorter precloacal tail length than adult males.

Subadult:

Both sexes possess at least three annuli. Subadult males have longer precloacal tail lengths than subadult females. Foreclaw length can be quite variable between the sexes at this stage and is an unreliable indicator of sex.

Juvenile:

Have two or fewer annuli. One cannot distinguish sex in juvenile turtles.

E. Marking the turtle

We will notch the marginal scutes to uniquely identify each individual.

When you hold the turtle with carapace up and its head at the 12:00 position, the first scute to the right of the small nuchal scute is the “A” scute, followed by “B” and “C” (Figure 4). The next four scutes comprise the bridge connecting the carapace and plastron and are not included in the naming scheme. After the bridge, the scutes go from “D” to “H” on the right side of the shell. The first scute on the left posterior side is “I.” The same naming scheme is used on the left side with the final scute (bordering the small nuchal scute at the 12:00 position) is “P” (Figure 4). We will start with a 3-letter code. Simply file a notch about one-half the way through the appropriate scute. I suggest writing the turtle’s code on its plastron with a permanent marker (See Figure 4). The written code will disappear when the turtle sheds its scutes, but having it written does help with data collection on recent recaptures.

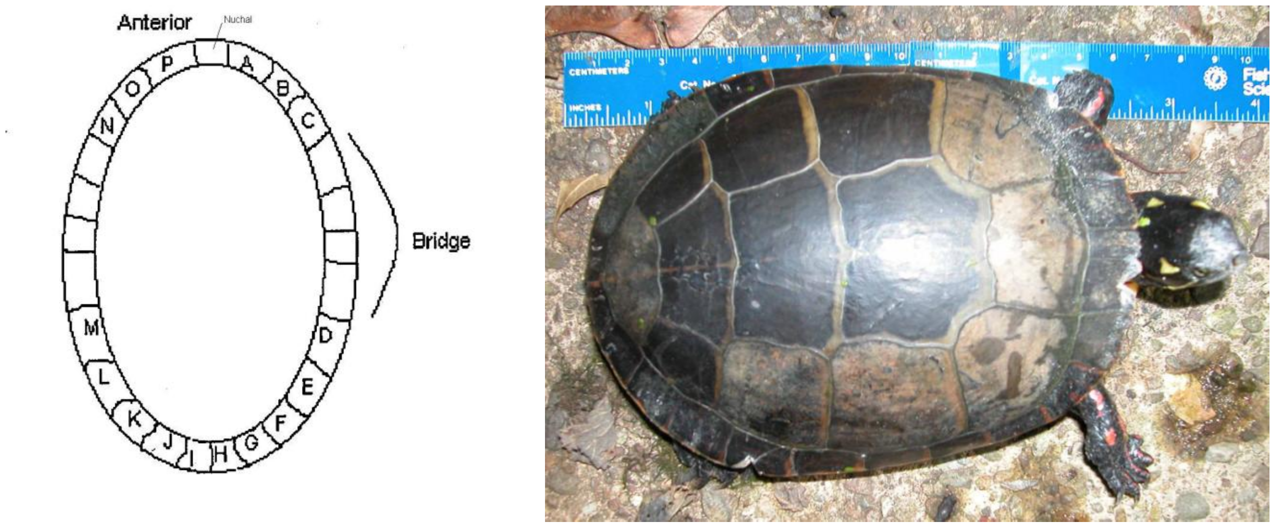

Figure 4. Schematic of turtle carapace with lettering scheme and actual carapace of an adult eastern painted turtle. This turtle's 3-letter code is ADN.

F. Releasing the turtle

After the turtle is measured and marked, release the turtle back into its habitat at a location close to the point of capture.

Literature Cited

Davis, D.E. and Winstead, R.L. 1980 Estimating the numbers of wildlife populations. *In* Wildlife Management Techniques Manual 4th edition, S.D. Schemnitz (ed). The Wildlife Society.

Freedberg, S. and Bowne, D.R. 2006. Monitoring juveniles across years reveals non-Fisherian sex ratios in a reptile with environmental sex determination. Evolutionary Ecology Research **8:**1499-1510.

Frazer, N.B., Greene, J.L. and Gibbons, J.W. 1993. Temporal variation in growth rate and age at maturity of male painted turtles, *Chrysemys picta*. Am. Midl. Nat., 130: 314–324.

Samuels, M.L. and Witmer, J.A. 2003. Statistics for the life sciences 3rd edition. Prentice Hall: Upper Saddle River, New Jersey.

Additional Faculty Notes

Site selection, setting and checking hoop traps

Any lentic habitat that supports freshwater turtles (other than snapping turtles) and contains sufficiently shallow areas to allow the setting of traps (~0.6 m deep, see below) is suitable for this study. Larger water bodies, however, are more difficult to adequately sample with a limited number of traps and time available. Therefore if more than one aquatic habitat is available, I suggest selecting the smaller one. If a large water body is the only one available, then definitely use it and cluster your traps in a smaller section (< 1 ha) of the habitat in order to increase the probability of recapture. In all cases, one should conduct preliminary trapping to determine what turtle species are present and if they are present in sufficient numbers – we want more than ten individuals (see below).

At Elizabethtown College, a large (1 ha) and small (.25 ha) pond are present on campus. Snapping turtles live almost exclusively in the larger pond and painted turtles live in the smaller one. My students hypothesize as to why this pattern exists but for the EREN study, I am only including turtle data from the smaller pond. Direct anthropogenic impacts on turtle species (i.e., collecting) may be greater in some ponds than others, but I do not think we can control for this during site selection. Fortunately, the snapping turtle is the most common species hunted for food and it will not be part of the study. Please contact me with specific questions about site selection. I realize that from a statistical design stand-point, this type of site selection is not ideal, but it may have to suffice – any suggestions are welcome.

Once you have a pond selected, it's time to start trapping. The turtle trap consists of three metal hoops (0.9 m diameter) attached by nylon netting (Figure 1). At one end of the trap is an opening that allows access to the trap. This opening is tapered so that captured turtles have difficulty escaping from the trap. I recommend reducing the size of this opening in order to reduce the probability of escape by weaving ¾" braided elastic through the netting encircling the opening. The opposite end of the trap is knotted with a length of rope. The end of this rope is used to stake the trap into the pond bottom and prevent the trap from being pulled into deeper water. The trap is kept rigid by two four foot poles (PVC or wood) extending parallel along the length of the trap (Figure 1). These poles are secured to the first and third hoop with metal clips. With the poles secured, the trap will not collapse and can be tossed into the aquatic habitat.

One should set the trap in approximately 0.6 m of water in order for the opening of the trap to be under water, but no greater than 0.9 m, or else the trap may be completely submerged, potentially drowning the trapped turtles. One should slightly open a can of sardines and place it in the rear of the trap, insuring that the can does not float. The rope at the rear of the trap should be staked into the pond's bottom.

A minimum of four (4) traps will be set. Each will be placed closed enough to the bank to meet the 0.6 m depth requirements. In small ponds (< 1 ha), the traps should be equally spaced to sample the entire habitat. In larger ponds (> 1 ha), the traps should be equally spaced within a subsection of the pond that approximately equals 1 ha. More intense sampling of a smaller area will increase the probability of recapture and is preferable to inadequate sampling of a large area. One trap can capture multiple individuals. We want at least 10 individuals of the same species per pond (Marchand and Litvaitis 2004).

Approximately 24 hours later, one should check each trap by grabbing hold of the first and third hoop and lifting the trap out of the water. If turtles are present, remove the stake and bring the trap onto the shore. With smaller turtles, one can reach into the rigid trap and pull out each turtle. Place each turtle into a bucket filled with water – each bucket can hold multiple turtles. For larger turtles, one might want to remove the support poles and collapse the trap around the turtle. If a snapping turtle is in the trap, I suggest collapsing the trap and allowing the snapping turtle to free itself. This may take several minutes but it is always successful. If both a snapping turtle and another species are in the trap, use one of the trap's hoops to immobilize the snapping turtle at one end of the trap and position the trap opening above the other turtle. Keep an eye on the snapping turtle as you reach in for the other one. If you are unable to immobilize the snapping turtle, then let the snapping turtle free itself first. The other turtle can wait. Once the turtles are removed from the trap, the trap should be reset at the original location. The same can of sardines should be used for both days of trapping.

Figure 1. Hoop trap containing two snapping turtles. The trap opening is located towards the left and the knotted rope is apparent in the bottom right corner.

Datasheet for turtles

Date: ____________ Pond: ____________

Status: New (N), recapture (R), or ID (turtle marked but doesn't match records)
Age-sex: adult male (AM), adult female (AF), Subadult male (SM), subadult female (SF), juvenile (J)
Carapace, plastron, precloacal: straight-line length (to 0.1 mm)
Foreclaw: Length of center nail on right foreclaw (to 0.1 mm)
Annuli: Number of growth rings on right abdominal scute

Trapper	Recorder	Status	TurtleCode	Age-sex	Species	Carapace	Plastron	PreCloacal	Foreclaw	Annuli	Comments

Curriculum Development Questions

Please review the following questions. We will discuss these at the EREN All Members meeting.

- What are the main challenges that you see for this project to be part of your laboratory teaching exercises?

- How does this project become multi-site/regional/continental as you teach it?

- How do we get students to interact across institutions?
  Ideas for breakout group leaders:

- How do we do data analysis that emphasizes the multi-site/regional/continental scale?

- How do we assess student learning and the effectiveness of the project?

- Are you familiar with other potential sources of data and/or projects that can complement existing EREN projects?